Dermatophytes Contain a Novel Lipid-Like Leukocyte Activator

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In the early phase of dermatophytosis, neutrophils are regularly detected microscopically in the infected skin. Although neutrophil recruitment may at least in part occur indirectly by complement activation, we asked whether dermatophytes might release chemoattractants for neutrophils. We cultivated various strains of different dermatophytes and tested fungal extracts for the presence of neutrophil chemotactic activity. As a result, we detected neutrophil chemotactic activity only in diethylether extracts, but not in aqueous extracts. We purified this lipid-like leukocyte activator (LILA) to apparent homogeneity by reversed-phase high performance liquid chromatography and found that purified LILA does not show ultraviolet absorption at wavelengths > 210 nm. Biologic studies revealed that LILA is as effective as formyl-methionyl-leucyl-phenylalanine in eliciting neutrophil chemotaxis, degranulation, and activation of the respiratory burst. Desensitization experiments in chemotaxis and degranulation with leukotriene B_4, platelet-activating factor, or 5-oxo-eicosanoids revealed that LILA does not cross-desensitize with any of these other lipid-like attractants and thus possibly acts via a distinct as yet postulated neutrophil receptor. It is hypothesized that LILA, similarly to formylated methionylpeptides in bacteria, represents a dermatophyte- and possibly fungus-specific lipid compound that allows the host phagocytes to specifically recognize fungal infection. This system would be similar to the recognition of bacteria by phagocytes via *N*-formylated methionyl-peptides, which represent a characteristic and unique system to identify bacteria. ***Key words: neutrophil/chemotactic factor. J Invest Dermatol 107:108–112, 1996***

Acute skin infections by dermatophytes are characterized by intensive inflammatory reactions. The factors inducing neutrophil accumulation in these inflammatory processes as yet are speculative.

Although leukotactic activities have been described in supernatants of bacteria (Schiffmann *et al*, 1975; Miyake *et al*, 1983; Marasco *et al*, 1984), there is only sparse information about leukotactic activity produced by dermatophytes: In the majority of studies it has been shown that different fungal species have serum-dependent chemotaxinogenic properties inducing liberation of leukotactic complement split products by complement activation (Cutler, 1975; Tagami *et al*, 1982; Davies and Zaini, 1983; Dahl and Carpenter, 1986).

There is only a single study showing that dermatophyte supernatants contained some direct leukotactic activity, which was not further characterized and which appeared to be of low molecular weight (Tagami *et al*, 1982). Preliminary results in our laboratory also indicated the existence of such factors (Brasch *et al*, 1991).

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Abbreviations: CB, complete buffer (PBS containing $CaCl_2$, $MgCl_2$ and 0,1% BSA); CI, chemotactic index; EC_{50} = 1U/ml, effective concentration of half-maximal chemotaxis; FMLP, formyl-methionyl-leucyl-phenylalanine; boc-MLP, *N*-tert-butoxycarbonyl-methionyl-leucyl-phenylalanine; LILA, lipid-like leukocyte activator; LTB_4, leukotriene B_4; MPO, myeloperoxidase; O_2^-, superoxide anion; PAF, platelet-activating factor; TFA, trifluoroacetic acid.

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In this study, we have reinvestigated supernatants of dermatophytes for neutrophil-chemotactic activity to determine if dermatophytes are capable of releasing leukotactic factors and thus are able to directly induce a leukocyte infiltration *in vivo*. Furthermore, we were interested to know whether proinflammatory mediators produced by dermatophytes elicit different functional responses in neutrophils and are distinct from as yet well-characterized inflammatory mediators.

MATERIALS AND METHODS

Leukocyte Preparation PMN for chemotaxis assays were freshly isolated from human venous blood as described previously (Henson, 1971; Kawohl *et al*, 1980).

Preparation of Extracts from Dermatophytes Five strains each of *Microsporum canis*, *Epidermophyton floccosum*, *Trichophyton rubrum*, and *Trichophyton mentagrophytes*, which had been isolated from infected human skin, were identified by their typical macroscopic and microscopic morphology on Sabouraud glucose agar. Subcultures were grown at 26°C on agar plates containing 4% glucose and 1% neopeptone (Difco Laboratories, Detroit, MI). After 2 wk, purity of these cultures was confirmed microscopically and by control cultures on Sabouraud agar. The mycelium was harvested from six plates of each strain, washed with phosphate-buffered saline (PBS), and smashed with glass beads of 0.5-mm diameter by use of a cell disintegrator (IMA, Zeppelinheim, FRG). The homogenate was centrifuged, and the cell-free supernatant was passed through a SepPak C^{18} cartridge (Waters Associates, Milford), which was subsequently eluted with methanol. The eluate was evaporated and resuspended in PBS for further purification.

Partial Purification of Chemotactic Factors from Cell Extracts by High-Performance Liquid Chromatography (HPLC) The liquid chromatography system consisted of a Spectra Physics chromatograph and a Kratos UV-Detector (Kratos, Westwood, NY).

Samples were applied to a reversed-phase C_{18} HPLC column (250 × 4.6 mm Nucleosil, 5 μm, Macherey and Nagel, Düren, FRG) previously

equilibrated with 0.1% trifluoroacetic acid (TFA) in water. Lipids were eluted with a gradient of increasing concentration of acetonitrile containing 0.1% TFA at a flow rate of 1 ml/min. Fractions were selected manually and were tested for their ability to induce myeloperoxidase release from PMN. Fractions containing biologic activity were rechromatographed using the same chromatographic system but with methanol and 0.15% (w/v) ammonium phosphate buffer, pH 4.5, as elution solvent.

Samples containing PMN-stimulatory activities were collected, evaporated, redissolved in PBS, and stored at temperatures below −70°C. Subsequently, samples of LILA were applied to a TSK-2000 exclusion HPLC column and were eluted with 0.1% TFA in water at a flow rate of 1 ml/min.

Determination of Chemotaxis and Chemokinesis For determination of neutrophil chemotaxis and chemokinesis experiments, a modified Boyden chamber assay system (Boyden, 1962) with indirect cell counting using β-glucuronidase as marker enzyme was used (Schröder *et al*, 1987).

To quantify the chemotactic activity, dose-response curves with aliquots of the separated LILA-HPLC fractions were established and used to determine EC_{50} doses. One EC_{50} dose (1 U/ml) is defined as that concentration of stimulus that leads to a half-maximal chemotactic response of PMN in the chemotaxis assay.

The maximal chemotactic activity (CI_{max}) of LILA in an optimal dilution for the chemotactic assay was expressed by the "chemotactic index" (CI), which is defined as the ratio of stimulated migration of PMN to the random migration of nonstimulated PMN and describes the chemotactic (or chemokinetic) efficacy of the stimulus used:

$$CI = \frac{\text{stimulated migration}}{\text{random migration}}$$

As a positive control we used defined chemotaxins at optimal concentrations, like 10^{-8} M LTB_4 (Paesel, Frankfurt, FRG) or 10^{-9} M FMLP (Sigma).

Chemokinetic activity was also determined by the use of the Boyden chamber technique. In a checkerboard analysis (Schröder and Christophers, 1989), panels of defined concentrations of LILA were placed in the upper and lower part of the Boyden chamber to stimulate chemokinetic migration of PMN.

Enzyme Release LILA-stimulated degranulation of PMN was determined using a modification of the method described by Preissner *et al*, 1983. As marker enzymes for azurophilic granules, myeloperoxidase activity (MPO, EC 3.11.1.7.) or β-glucuronidase activity (EC 3.2.1.31.) were determined.

Desensitization Experiments Cross-reactivities of chemotaxins to PMN–receptor-dependent functional activation were studied by preincubating 10^7 PMN with different chemotaxins at optimal concentrations as determined previously (2 U of LILA per ml, 2×10^{-8} M LTB_4, 300 ng of PAF per ml, 10^{-5} M BocMLP) for 20 min at 37°C (Preissner *et al*, 1983). After a subsequent incubation of cells with 5 μg of cytochalasin B per ml for 5 min, cells were stimulated with a second chemotaxin (1.5 U of LILA per ml, 15 ng of LTB_4 per ml, 200 ng of PAF per ml, 4×10^{-8} M FMLP). After 30-min incubation, the released β-glucuronidase activity was determined in supernatants as described above.

Superoxide Anion (O_2^-) Production Superoxide anion production was measured using a modification of the method described by English *et al* (1981). Briefly, 250 μl of a neutrophil suspension [10^7 cells per ml of PBS containing 0.1% glucose (w/v)] were pretreated with 5 μg of cytochalasin B per ml (Sigma, Munich, FRG) for 5 min at 37°C, and thereafter 500 μl of prewarmed (37°C) oxidized cytochrome C solution (horse cytochrome C in 40 nM PBS, Sigma) were added. Finally, LILA at various concentrations and PBS or FMLP were added and the mixture was incubated for an additional 30-min period at 37°C. Cellular responses were stopped by chilling cell-containing vials in ice water followed by centrifugation of cells.

Supernatants were measured at two wavelengths (E_1 = 474.4 nm, E_2 = 549.1 nm; Hitachi 150-20 double-beam spectrophotometer) and O_2^- concentration was calculated as the quotient E_2/E_1 and given as nanomoles of reduced cytochrome C. For calibration, totally reduced (with sodium dithionite) and totally oxidized (with K_3 [$Fe(CN)_6$]) cytochrome C were used.

Physical Characterization of LILA Aliquots of LILA in CB (complete buffer: PBS containing $CaCl_2$, $MgCl_2$, and 0.1% BSA) were either subjected to 100°C for 5 min, stored at 60°C for 40 min, and treated with 10 cycles of freezing (−80°C) and thawing (37°C), or adjusted to various pH values from 2.5 to 10.0 by HCl or NaOH. Thereafter, all samples were adjusted to pH 7.2 in PBS, and osmolarity and chemotactic activity (U/ml) were compared to an untreated control.

LILA, dissolved in diethylether, was extracted either with PBS at pH 7.3, with 0.1 M citrate buffer, pH 5.0, or with 0.1 M $NaHCO_3$, pH 8.0. Aliquots of the ether phases and the three buffer phases (pH 5.0, 7.3, and 8.0) were lyophilized, dissolved in CB, and tested at various dilutions for MPO release activity.

Three identical experiments with LILA samples of one strain each of *M. canis* and *T. mentagrophytes* were performed.

UV spectra of partially purified LILA samples were obtained in methanol using a double-beam UV spectrophotometer (Hitachi 150-20).

RESULTS

Identification and Partial Characterization of PMN Chemotactic Lipids in Supernatants of Lysed Dermatophytes Supernatants from homogenized mycelia of different dermatophytes contain chemotactic activity for neutrophils, which was detected by the Boyden chamber chemotaxis assay. In order to examine the physicochemical properties of this leukocyte chemotactic activity, mycelia extracts were depleted from lipids by the use of solid reversed-phase extraction cartridges. Bound lipids were stripped from the cartridge by the use of methanol.

Both the lipid-depleted crude extracts as well as the lipid-containing fractions were examined for neutrophil chemotattractants. Chemotactic activity for neutrophils was identified exclusively in the lipid extracts, whereas non–lipid-like compounds remaining in the aqueous effluent of the reversed-phase cartridge did not show significant chemotactic activity.

LILA from dermatophytes were partially purified by the use of reversed-phase (RP) HPLC: Using a gradient of acetonitrile in water containing TFA for elution, peak neutrophil chemotactic activity was eluted at approximately 75% acetonitrile/TFA **(Fig 1)** in all extracts of different dermatophytes containing LILA.

Little variation of retention time was detected in different LILA preparations obtained from different strains of dermatophytes.

Out of 20 tested strains of *M. canis*, *E. floccosum*, *T. rubrum*, and *T. mentagrophytes*, 18 were found to contain a stimulatory activity. Extracts of two *T. rubrum* strains did not contain measurable neutrophil chemotactic or degranulation-inducing activity.

M. canis strains were found to produce the highest amounts of LILA **(Table I)**. In addition, when LILAs obtained from different sources were coinjected in a RP-HPLC system, chemotactic activity eluted in a single peak of biologic activity (data not shown).

Although HPLC fractions containing partially purified LILA showed UV-absorbing (>215 nm) peaks, these were not found when activity was further purified by RP-HPLC. Indeed, some LILA fractions were obtained that revealed no UV absorbance at wavelengths higher than 210 nm (in acetonitrile, data not shown).

Partially purified LILA was tested under various conditions for stability of biologic activity. Boiling for 5 min as well as treatment at 60°C for 40 min in PBS, pH 7.2, did not alter chemotactic activity (number of Units or efficacy (percentage of migrating cells)) when compared with a control solution stored for 30 min at 4°C. Also, 10 cycles of freeze/thawing did not alter chemotactic activity. Thirty percent of activity was lost when LILA was incubated for more than 2 h at pH 10 or 2.5. Surprisingly, storage of LILA in acidic methanol (pH 4.0) for 24 h at 4°C resulted in a drastic (68%) loss of activity.

Leukotactic activity was extractable from aqueous solutions with diethylether in the pH range 2 through 9. Size-exclusion HPLC, using a TSK-2000 HPLC column and partially purified LILA revealed, PMN chemotactic activity in fractions corresponding to $M_r < 500$ (data not shown).

Because the biologically active fractions isolated from different strains and species showed nearly identical chromatographic purification characteristics, we used a representative *M. canis*-LILA preparation for all further biologic and physical experiments.

When structurally defined lipophilic neutrophil chemotaxins such as LTB_4, C_{16}-PAF, or two 5-oxo-eicosanoids were applied to the chromatography systems described above, all chemotactic substances showed different chromatographic behavior from that of

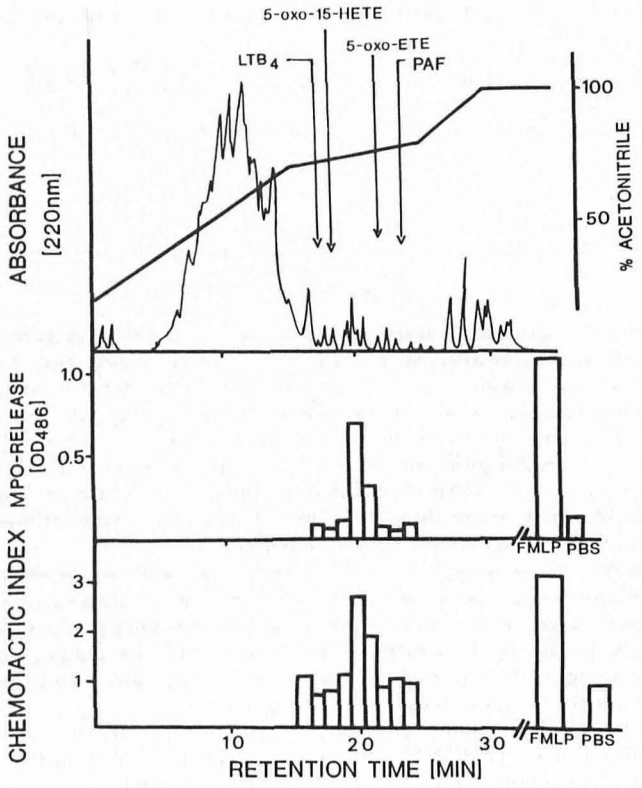

Figure 1. Identification of LILA in dermatophyte extracts. *M. canis*-derived lipid extracts were separated on a reversed-phase (RP-18) HPLC column using a gradient of acetonitrile in TFA-containing water. In the effluent, absorbance at 220 nm was monitored (*A*). Fractions were analyzed for MPO-releasing activity (*B*) and PMN-chemotactic activity (*C*). FMLP served as a positive control for MPO release at 1.6×10^{-6} M and at 3×10^{-9} M for PMN chemotaxis, whereas CB served as a negative control. For comparison, authentic samples of LTB_4, 5-oxo-15-HETE, 5-oxo-ETE, and PAF (C_{16}) were chromatographed using identical conditions. The elution position of each of these compounds is indicated by the *arrows*. LTB_4 as well as 5-oxo-eicosanoids were detected due to their UV absorption at 280 nm, whereas PAF was detected via neutrophil chemotactic activity. A typical HPLC run is shown.

fungal LILA **(Fig 1)**. Whereas PAF and 5-oxo-ETE constantly eluted later than LILA, LTB_4-derived activity as well as 5-oxo-15-HETE eluted earlier than LILA **(Fig 1)**. Moreover, PAF application resulted in only 20% recovery of chemotactic activity from the RP-18 HPLC column when acetonitrile containing 0.1% TFA was used as eluent.

Table I. LILA Content of Different Species and Strains of Dermatophytes

Species	LILA Content[a]				
	Strain 1	2	3	4	5
M. canis	1250	375	827	419	+[b]
T. rubrum	0[c]	75	0[c]	100	150
T. mentagrophytes	20	424	50	32	+[b]
E. floccosum	75	120	300	110	57

[a] For each strain, mycelium grown on six plates was extracted, and extracts were separated on two RP-18 HPLC columns as detailed under *Materials and Methods*. The number of Units of chemotactic activity was determined from the biologically active fractions by testing fractions with at least six different dilutions and calculation of the EC_{50}.

[b] Fractions contained LILA. Number of Units not determined.

[c] No chemotactic activity was found.

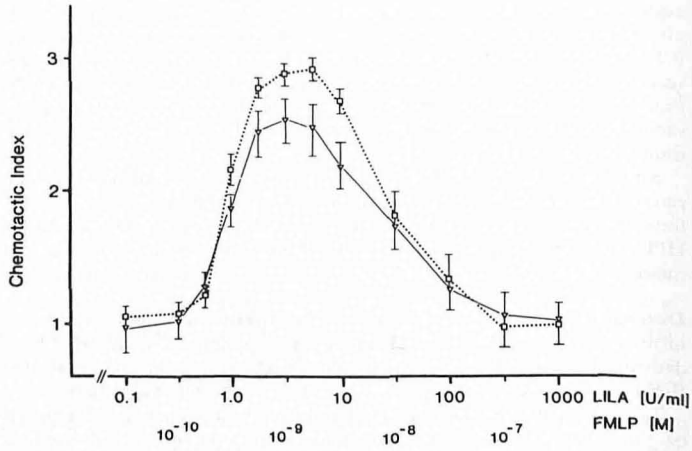

Figure 2. Comparison of PMN-chemotactic activity of LILA and FMLP. PMN-chemotactic activity of a partially purified LILA preparation (▼) and FMLP (□) were tested for concentration dependency of chemotactic activity using the indirect cell-counting method as detailed under *Materials and Methods*. Results are expressed as mean ± SD of three experiments each performed in duplicate.

LILA Stimulates Neutrophil Chemotaxis and Chemokinesis When partially purified LILA preparations were analyzed for neutrophil chemotactic activity in the Boyden chamber assay system, a concentration-dependent increase in the numbers of indirectly counted neutrophils in the lower part of the Boyden chamber was found **(Fig 2)**. Similarly, chemotactic activity was identified in LILA preparations when the number of cells sticking to the lower filter side was determined microscopically in a modified Boyden chamber assay system (data not shown).

A maximum chemotactic index (CI_{max}) of 2.6 ± 0.3 was found when all LILA preparations of the 18 active strains were tested in the indirect cell-counting chemotaxis assay. FMLP gave a CI_{max} value of 2.9 ± 0.1 when a concentration of 10^{-9} M was used under the same conditions. Therefore, LILA appears to have a similar efficacy (percentage of input migrating cells) as FMLP. A characteristic dose-response curve of the PMN-chemotactic properties of LILA is shown in **Fig 2**. The concentration necessary to elicit half-maximal responses was defined as 0.1 Unit per chamber, which corresponds to 1 Unit per ml. Interestingly, the dose-response relation of LILA-dependent neutrophil chemotaxis shows a marked similarity to that elicited by FMLP, giving a typical bell-shaped curve indicating a loss of chemotactic activity at high concentrations of LILA **(Fig 2)**.

In order to investigate whether LILA-derived locomotory responses are chemotactic or rather chemokinetic, a checkerboard analysis was performed. As shown in **Table II**, LILA also expresses chemokinetic activity for neutrophils, apart from chemotactic responses. The chemokinetic efficacy (percentage of migrating cells), however, is lower than that of chemotaxis, indicating that LILA acts mostly as a chemotactic factor. Half-maximal chemokinetic activity occurs at the same concentration as chemotactic activity **(Table II)**.

LILA Is a Secretagouge for Neutrophils Well characterized chemotaxins are known to induce the release of lysosomal PMN constituents when cells are pretreated with cytochalasin B. Therefore, we investigated whether LILA has similar biologic properties. As shown in **Fig 3**, LILA induces the release of the azurophilic granule constituent myeloperoxidase (MPO) as well as of β-glucuronidase in a concentration-dependent fashion when cells were pretreated with cytochalasin B. In the absence of cytochalasin B, no significant MPO release or β-glucuronidase release occurred (data not shown). The maximal release of β-glucuronidase (as percentage of a total control) is comparable to that detected after FMLP stimulation **(Fig 3)**. When compared with the chemotaxis dose-

Table II. Checkerboard Analysis of PMN-Locomotory Responses Toward LILA[a]

LILA Concentration in the Lower Chamber[b]	LILA Concentration in the Upper Chamber[b]					
	0	0.6	1.25	2.5	5	10
0	**1.0**	1.0	1.0	0.9	1.0	1.1
0.6	1.5	**1.2**	1.3	1.0	1.1	0.9
1.25	2.2	1.6	**1.6**	1.5	1.3	1.1
2.5	2.6	2.2	2.1	**2.0**	1.5	1.3
5	3.0	2.8	2.4	2.4	**1.8**	1.5
10	2.9	3.0	2.6	2.4	2.0	**1.6**

[a] Values represent means of calculated chemotactic indices from three determinations performed in duplicates using the indirect cell counting Boyden chamber technique as detailed under *Materials and Methods*. SD did not exceed 11% of the mean. Bold numbers represent values of experiments performed with identical LILA concentrations in the upper and lower part of the chamber.

[b] Concentrations represent the number of Units LILA per chamber.

response curve **(Fig 2)**, half-maximal enzyme release occurred at nearly 8-fold higher concentration.

LILA does not cross-desensitize PMN activation with other chemotaxins. Furthermore, LILA is a very effective agent in eliciting the respiratory burst, in particular superoxide-anion production, of neutrophils. As shown in **Fig 3**, half-maximal O_2^- production occurred at similar LILA concentrations as for enzyme release. O_2^- production is similar to that with FMLP as stimulus **(Fig 3)**.

Well known chemotactic factors elicit enzyme release by binding to ligand-specific membrane receptors, which are desensitized by pretreatment of the cells with ligand or homologues. This results in a strongly diminished or absent response to a second challenge with these ligands, but not with other stimuli. This system reveals some indication, whether a chemotaxin induces functional responses via unknown receptors or via known receptors for well characterized chemotaxins. Therefore, PMN were preincubated with a panel of defined chemotaxins or with the nonchemotactic FMLP antagonist BocMLP, respectively. Under these conditions cells do not release azurophilic granule constituents (data not shown). After subsequent preincubation with cytochalasin B and a second challenge with various chemotaxins, the resulting enzyme release was determined. As shown in **Table III**, there was no inhibition of LILA responses by BocMLP at concentrations that inhibit degranulation by FMLP.

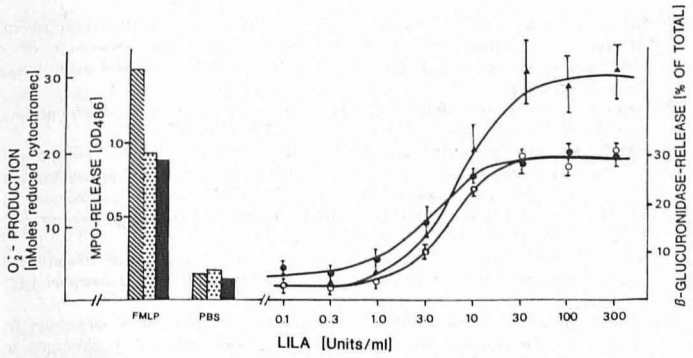

Figure 3. LILA induces degranulation and O_2^- production in PMN. Neutrophils pretreated with cytochalasin B were tested with LILA for its ability to release azurophilic granule constituents as well as to induce superoxide-anion production upon stimulation with various concentrations of LILA. As markers for degranulation, the azurophilic granule constituents myeloperoxidase (○) and β-glucuronidase (●) were used. O_2^- production (▲) was measured via superoxide dismutase–inhibitable cytochrome C reduction. 10^{-7} M FMLP and PBS served as controls for cytochrome C reduction (▨), whereas for myeloperoxidase release, 10^{-8} M FMLP and PBS (▩) were used as controls. Similarly, 10^{-8} M FMLP and PBS (■) served as controls for β-glucuronidase release. Results shown represent the mean of eight different LILA preparations ± SD.

Table III. Desensitization of PMN Enzyme Release by LILA and Other Chemotaxins[a]

Preincubation With	Stimulation With			
	LILA (15 U/ml)	LTB_4 (15 ng/ml)	PAF (200 ng/ml)	FMLP (4×10^{-8} M)
LILA (20 U/ml)	**29**	51	88	95
LTB_4 (20 ng/ml)	90	**18**	79	106
PAF (300 ng/ml)	115	41	**21**	96
BocMLP (10^{-5} M)	88	98	84	**11**
Buffer-control	100	100	100	100

[a] Enzyme release (β-glucuronidase) was determined in chemotaxin-preincubated PMN after subsequent stimulation with different chemotaxins at given concentrations. Results are expressed in percentage of net enzyme release of control (buffer-preincubated cells). Values represent means of three duplicate experiments with LILA from *M. canis* and *T. mentagrophytes*. SD did not exceed 18% of the mean. Note autologous desensitization of each chemotaxin (bold numbers).

In contrast, preincubation of cells with LILA affected enzyme release induced by LILA itself. To a lesser degree, cells stimulated with LTB_4 were also affected. When other factors were used as a second stimulus, no reduction or inhibition of enzyme release occurred **(Table III)**. Preincubation of PMN with LTB_4 or PAF did not influence LILA-induced enzyme release; however, preincubation with PAF decreased LTB_4-induced enzyme release in addition to that by PAF. PMN enzyme release induced by lipid-like factors such as LILA, LTB_4, or PAF was not altered by the FMLP-receptor antagonist BocMLP.

DISCUSSION

Dermatophyte-derived neutrophil chemotactic activity may be attributable to one or more lipids that are functionally and structurally closely related or identical, as indicated by HPLC analyses and the characteristics of their biologic activity. In attempts to purify LILA to homogeneity, we found that LILA apparently does not show UV absorption of >210 nm. Thus, known lipid-like attractants such as LTB_4, some of the hydroxy-eicosatetraenoic acids (HETEs), as well as 5-oxo-eicosanoids, all of which have characteristic UV absorbance profiles between 230 and 280 nm (Goetzl and Pickett, 1980; Samuelsson *et al*, 1987; Schwenk *et al*, 1992; Schwenk and Schröder, 1995), are not responsible for LILA activity. In addition, PAF, which like LILA does not show a characteristic UV absorbance profile, can be excluded as being responsible for lipid-like chemotactic activity, since its elution pattern, extraction pattern, and stability are different from those of LILA.

Our attempts to purify LILA to homogeneity and to identify its chemical structure have been hampered so far. This may be a result of the lack of material as well as difficulties in purification. So far, problems were caused mainly by the sensitivity of LILA toward treatment with methanol, a solvent necessary for purification by HPLC methods. This behavior is unique for this mediator.

Most strains of the dermatophytes tested contained LILA, which was identified by its identical chromatographical behavior upon RP-HPLC for all species. We were unable to identify a similar activity in supernatants of bacteria or in lipid extracts from normal skin or heel callus when a similar purification protocol was used. Therefore, LILA appears to be a rather dermatophyte-specific lipid.

It is tempting to speculate that LILA may represent a dermatophyte-derived compound that allows the host phagocyte system to recognize dermatophyte infections and thus to initiate inflammatory defense mechanisms against these infectious pathogens. A similar system was originally postulated 20 years ago for bacteria (Schiffmann *et al*, 1975). Later it was shown that *N*-formylmethionyl peptides, which are exclusively released from bacteria but not from eukaryotes, are strong inducers of phagocyte defense mechanisms including leukocyte-chemotaxis, degranulation, and activation of the respiratory burst (Schiffmann *et al*, 1978; Showell *et al*, 1976; Marasco *et al*, 1984).

Proinflammatory properties (leukotactic activity, induction of

PMN degranulation, superoxide anion production) of dermatophyte-derived neutrophil chemotaxins interestingly were found to be of similar efficacy as known for well characterized chemotaxins such as FMLP or LTB_4. In addition, dose-response curves for LILA-dependent PMN chemotaxis were found to be bell-shaped **(Fig 2)**, a characteristic seen in all as yet well characterized PMN chemotactic factors (Showell *et al*, 1976; Schröder *et al*, 1990). The reasons for the decrease in chemotactic responses *in vitro* at high concentrations are still speculative. It has been suggested that an increased adherence of PMN to the upper filter surface results in a diminished migratory response (Fehr and Dahinden, 1979).

In addition to chemotactic responses, LILA also provokes chemokinetic PMN responses. Although the chemokinetic index is slightly lower than in the chemotaxis experiments, optimal stimulation for both chemotaxis and chemokinesis occurs at the same concentrations of LILA. This property is also found in experiments performed with many other PMN chemotaxins such as LTB_4, FMLP, C5a, or IL-8 (Schröder *et al*, 1987; Schröder and Christophers, 1989; Schröder *et al*, 1990).

In addition to physicochemical data, results from biologic experiments further strengthen the hypothesis that dermatophyte-derived LILA is an unknown PMN chemotaxin: Cross-desensitization studies **(Table III)** revealed that LILA exerts a strong desensitization of LILA-dependent responses only when PMN are preincubated with LILA, but not after preincubation with other chemotaxins. Since the FMLP-receptor antagonist BocMLP (Schiffmann *et al*, 1975) did not affect LILA-dependent enzyme release, the idea is supported that LILA does not elicit functional activation via the FMLP receptor. Interestingly, preincubation of PMN with LILA results in reduced degranulation when LTB_4 is used as a second stimulus, but not *vice versa* **(Table III)**. This unexpected phenomenon could be explained either by receptor class desensitization (Didsbury *et al*, 1991), in which case we postulate a separate PMN membrane receptor for LILA, or alternatively by induction of LTB_4 production during the preincubation period. Similar results were obtained when PAF was used for preincubation of PMN, confirming previous results (Schröder *et al*, 1990). Receptor class desensitization (Didsbury *et al*, 1991) or a production of LTB_4 during the incubation period would explain this effect as well (Schröder *et al*, 1994).

Therefore, our data could indicate the presence of membrane receptors for LILA on neutrophils, which seem to be distinct from those specific for F-Met peptides, LTB_4 and PAF.

Although in principle LILA could also act via the very recently postulated 5-oxo-eicosanoid receptor (Schwenk *et al*, 1992; Powell *et al*, 1994), this is unlikely, because in contrast to other well-defined chemotaxins, 5-oxo-eicosanoids do not elicit enzyme release in either eosinophils (Schwenk and Schröder, 1995) or neutrophils (data not shown).

Cross-desensitization experiments with LILAs obtained from different species of dermatophytes revealed strong desensitization, indicating that different dermatophyte species produce the same or structurally related LILAs. It is likely but not yet fully proved that similar lipid-like chemotactic factors are also produced by *Candida albicans* (Cutler, 1975; Brasch *et al*, 1990; Brasch *et al*, 1992). Thus, chemotactic lipids produced by dermatophytes and pathogenic yeasts may be structurally related. LILAs could represent a fungus-specific recognition signal for the phagocyte system of the host to prevent fungus infection, similar to *N*-formylated methionyl peptides (Schiffmann *et al*, 1975; Marasco *et al*, 1984; Rot *et al*, 1987), which seem to function as a bacteria-specific recognition principle for host-phagocytes.

LILAs are attractive candidates to explain the inflammatory response often found in fungal infection. Since these mediators are pathogenic, further purification and structural characterization is necessary to gain insight into the importance of LILAs *in vivo*. Based on the idea that other fungal species may contain similar or identical LILAs as characteristic cellular constituents, work is in progress to analyze the chemical structure of LILA and LILAs from additional species of pathogenic fungi.

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